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Isolation of captan from *Cyrtanthus suaveolens*: the effect of pesticides on the quality and safety of traditional medicine

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Bioassay-guided fractionation of dichloromethane extracts of *Cyrtanthus suaveolens* using the *Salmonella*/microsome assay (strain TA98) led to the isolation of captan [*N*-(trichloromethylthio)cyclohex-4-ene-1,2-diccarboximide] at a concentration of 100mg kg^{-1} of dry plant material. Captan is a pesticide used on a large scale on agricultural and horticultural crops. It is known for its mutagenic, genotoxic and teratogenic effects. Issues of quality and safety of traditional medicinal plants grown commercially in South Africa and the need for the establishment of good agricultural practices are discussed.

Introduction

Cancer is one of five major causes of death in developed countries (Rang *et al*. 1995). The most common causes of cancer are from environmental effects and exposure to certain chemical compounds. These include insufficiently tested medicine, environmental pollutants and food contaminants (Pozharskii *et al*. 1997). Tremendous care is taken in the monitoring of environmental pollutants, food additives, pharmaceutical products and various chemicals of agricultural importance such as pesticides for possible carcinogenic and/or mutagenic effects (Pozharskii *et al*. 1997). These efforts result in the withdrawal of many products whether of nutritional, pharmaceutical or agricultural importance from the market especially in the developed countries.

Plants used in traditional medicine provide primary health care to the majority of people in developing countries (Addae-Mensah 1992). These plants are presumed to be safe due to their long-term use for the treatment of various ailments, by different ethnic groups. However, research showed that crude extracts of plants used in traditional medicine are a source of potential carcinogens (De Sá and Ferrão Vargas 1999). These plants need to be thoroughly investigated, in the interests of patient safety.

A recent study on the mutagenic effects of some plants used in South African traditional medicine revealed mutagenicity of different parts of *Cyrtanthus falcatus* and *C. suaveolens* in the *Salmonella*/microsome assay (strainTA98) (Elgorashi and Van Staden 2004). The primary objective of this study was to isolate the mutagenic compound(s) from the crude extracts of *C. suaveolens*. However, the isolation of Captan, a known fungicide, from the plant extracts raises the importance of issues of quality and safety of medicinal plants produced commercially for the South African market and the need for good agricultural practices to be established.

Material and Methods

Plant material

Plants of *C. suaveolens* were obtained from Green Goblen Nursery, Durban. A voucher specimen (Elgorashi NU6) was deposited at the Herbarium of the University of Natal Pietermaritzburg. Mr G Duncan, of the National Botanical Institute, confirmed the identity of the plants extracted.

Extraction and isolation

Extraction and isolation were achieved by bioassay-guided processes using *Salmonella typhimurium* TA98. Dried (oven at 55°C) and powdered underground parts (bulbs/roots) of *C. suaveolens* (250g) were extracted with dichloromethane (1.25l x 2) for 24h to give 3.13g of crude extract. The crude extract was fractionated using a column packed with silica gel 60 (70–230 mesh). The column was eluted with hexane and then with hexane mixed gradually with 1%, 2%, 5%, 10%, 12%, 20%, 40%, 50% and 60% ethyl acetate, ending with 100% ethyl acetate.

Fractions of 20ml were collected and examined using thin layer chromatography. Fractions of similar content were combined and tested for mutagenic effects. Fractions 128–137

(672mg) were combined and developed on a column using silica gel eluted with the same solvent systems. Fractions of 20ml were collected. Fractions 98–124 were combined (200mg) and developed on silica gel 60 F_{254} preparative thin layer chromatography plates (2mm) using hexane: dichloromethane:methanol (14:8:1) to give captan (25mg).

Mutagenicity assay

A mutagenicity test was carried out using the *Salmonella/*microsome assay based on the plate-incorporation procedure with *Salmonella typhimurium* tester strain TA98. The assay was performed according to Maron and Ames (1983). Stock (100µl) bacteria in 20ml Oxoid nutrient broth No. 2 were incubated for 16h at 37°C. The bacterial cultures (100µl) were added to 100µl of the plant extract in 500ml phosphate buffer and 2ml of agar containing biotin-histidine (0.5mM). The mixture was poured onto a minimal agar plate and incubated at 37°C for 48h. 4-Nitroquinoline-*N*-oxide (4 NQO) was used as a positive control at a concentration of 2µg ml^{-1}. Samples were tested in triplicate with two replicates. Three dilutions were prepared per sample.

Results and Discussion

Bioassay-guided fractionation of the crude extracts of *C. suaveolens* using the *Salmonella/*microsome assay led to the isolation of captan (Figure 1). Its NMR and EIMS data are in agreement with those reported for captan (Angioni *et al*. 2003). Results on the mutagenic effect of captan at different stages of purification are presented in Table 1. Captan has been used commercially on a large scale, for many years, in horticulture and agriculture as an antifungal or bacteriostatic agent (Bridges 1975). This compound induces base-pair substitution mutations in *Escherichia coli*, *Salmonella typhimurium*, *Neurospora crassa* and Chinese hamster cells. It also causes gene conversion in *Saccharomyces cervisiae*, chromatid breaks in heteroploid human embryonic lung cells and lethal mutations in rats and mice (Ficsor *et al*. 1977, Vigfusson and Vyse 1980).

The use of herbal medicine in seeking cures from illness can be traced back over five millennia to documents produced by early civilizations worldwide (Hamburger and Hostettmann 1991). In South Africa and most developing countries traditional medicine still forms the backbone of rural medical practice. About 3 000 plant species are used as medicine in South Africa alone and of these about 350 species are commonly used and traded in large volumes (Van Wyk *et al*. 1997). The majority of these plants have been and, where still possible, are harvested from wild populations within South Africa and neighbouring countries. This results in their overexploitation. To add to this problem of overexploitation, a large proportion (±85%) of the plant material traded consists of non-renewable material, material which when harvested either severely damages the plant or kills it, such as bulbs, rhizomes and bark (Mander 1997).

This excessive pressure on wild plant populations had resulted in many species becoming extremely rare and even nonexistent outside protected areas. Heavy exploitation of *Warburgia salutaris*, *Cassine transvaalensis*, *Alepidea amatymbica* and *Erythrophleum lasianthum* were recorded as early as 1938 (Gerstner 1941). It is clear that with the existing demand for medicinal plants sustainable harvesting is not feasible. If the future demand is to be met, it is essential that many of the popular species utilised in traditional medicine be developed for commercial cultivation.

There are two potential solutions, the production of plant materials by community nurseries or small-scale rural farmers and large-scale production by commercial farmers. However, before commercial farms can venture into the propagation of indigenous medicinal plants, cultivation, handling and processing protocols have to be developed.

Cultivation of medicinal plants encompasses a number of agricultural practices including sowing, irrigation, fertilisation, pesticide and herbicide application, harvesting and processing. These agricultural practices may cause the contamination of plant material with fertilisers and pesticide residues, which accumulate from application during cultivation and administration of fumigants during storage. Medicinal plants can not be grown in soils that are contaminated by sludge and heavy metals. Other fertilising agents

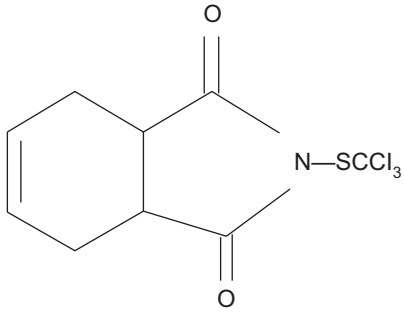

Figure 1: Chemical structure of captan

Table 1: Number of his+ revertants in *Salmonella typhimurium* strain TA98 produced by captan at different stages of purification

Extract No.	No. of colonies						
	Extract Concentration (µg ml)					+ve control	-ve control
	5 000	1 000	500	100	50		
1	752 ± 39		137 ± 7		29 ± 6	252.6 ± 18.9	21.4 ± 3.9
2	UC		816 ± 31		252 ± 23	179 ± 0.7	24 ± 6.3
3	–	559 ± 20		277 ± 15	35 ± 5	132 ± 13.4	21.8 ± 3.3
Captan		742 ± 79		143 ± 16	31 ± 7	239 ± 8.3	24.7 ± 1.3

Extracts induced revertant colonies numbering at least twice the revertant control number were mutagenic
UC = uncountable colonies

should be applied sparingly and in accordance with the demands of the plant species.

Pesticide and herbicide application should be avoided as far as possible. If their application is inevitable, pesticides and herbicides application should be according to quality control methods for medicinal plant materials issued by the World Health Organization (WHO). These quality control methods stressed that the toxicological evaluation of pesticide residues in medicinal plant materials should be based on the likely intake of the material by patients. In general, the intake of residue from medicinal plant material should account for not more than 1% of total intake from all sources, including food and drinking water (WHO 1998).

In South Africa there is little regulation and development of the medicinal plant trade despite its economic, conservational and cultural importance. However, there are two important provisions in the South African Medicines and Medical Devises Regulatory Bills: the first regulates general traditional healers in South Africa under the associated Health Service Professions Act of 1982 as amended. The regulation entitles medical providers to practice for gain and call themselves members of that profession. In accordance with the second, issues of safety and quality take precedence over demonstrations of efficacy (WHO 2001).

Although the above two regulations acknowledge the role of traditional healers in the health care system and the importance of safety and quality of plants used in traditional medicine, they fall short of calling for Good Manufacturing Practice (GMP), initiated by the WHO in the mid-eighties with the ultimate aim of ensuring continuous high quality production of products for human consumption and the guidelines for the good Agricultural Practice of Medicinal and Aromatic herbs which is intended to apply to the growing and primary processing of all such plants traded and used in the European Union (Franz 1989, Schilcher 1989).

If the development of a safe and high quality medicinal plant industry in South Africa is to occur it is important that sound guidelines for production, processing and marketing are developed that can be implemented from the onset. Some common ground between those currently used in Europe and the rest of the world and those to be implemented in South Africa should be reached, particularly where export potential is envisaged.

Above all, if plants are to be cultivated for the South African traditional market then several cultural aspects have to be taken into consideration. Conservative traditional healers believe that plants grown under western agricultural conditions (i.e. with fertilisers and in straight lines) will not have the same medicinal properties as those harvested from the wild. This is a 'supernatural' phenomenon and therefore scientific validation of the biological activity of cultivated plants will not sway traditional healers beliefs. However, the dwindling plant numbers coupled with the increased demand for these plants has resulted in large price increases and limited supplies of certain species. This means that most healers are willing to accept that cultivation is the only solution (Stafford 2003).

This study also raises concern where reporting on bioactivity of plant crude extracts without the isolation and identification of the active compounds. The activity of these extracts might be due to a contaminant, such as pesticide, rather than a plant product, which may lead to incorrect conclusions.

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